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High Molecular Weight Neutrophil Chemotactic Factor: Recognition, Characterization, and Role in the Deactivation of Neutrophilic Leukocytes

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Idiopathic acquired cold-induced urticaria has provided a model to study release of mast cell-derived chemical mediators into the blood and alterations of neutrophilic leukocyte motility. A factor chemotactic for neutrophilic leukocytes appeared in the circulation after local experimental challenge with ice. After partial purification by Sephadex G-200 gel filtration and by anion and cation exchange chromatography the neutrophil chemotactic activity was excluded on Sepharose 4B gel filtration, indicating a molecular weight in excess of 750,000. On isoelectric focusing it exhibited a neutral isoelectric point. This chemotactic factor showed preferential chemotactic activity for neutrophils and deactivated these cells *in vitro* and *in vivo*. HMW-NCF may prove to be a useful marker of mast cell activation and its release may modulate the capacity for motility of neutrophilic leukocytes in humans.

Patients with the disorder of acquired cold-induced urticaria/angioedema have provided an experimental model for the study of mast cell mediator release and of leukocyte motility [1, 2]. Evidence for involvement of the mast cell in this disorder is derived from the ability of patients' serum or IgE to transfer cold urticaria to the skin of a normal person [3] as well as from finding histamine and a low molecular weight eosinophil chemotactic factor, comparable in size to the eosinophil chemotactic factor of anaphylaxis (ECF-A), in the venous blood after local challenge with ice [4–6]. Augmented neutrophil chemotactic activity has been recognized in the venous blood of such patients after local ice challenge [1]. This neutrophil chemotactic activity has a high molecular weight, a neutral isoelectric point, and the ability to alter the motility of neutrophilic polymorphonuclear leukocytes; it has been designated high molecular weight neutrophil chemotactic factor (HMW-NCF).

EXPERIMENTAL PROTOCOL

Patients with idiopathic acquired cold-induced urticaria, after giving informed consent, participated in a protocol in which one arm was immersed in ice water while the control arm remained at room temperature. Indwelling intravenous needles were placed in both arms at the antecubital fossae, and blood was obtained from both just before and at timed intervals after immersion of one arm. Serum was assessed for neutrophil chemotactic activities in a modified Boyden chamber assay [7]. Peripheral blood neutrophilic leukocytes were obtained for analysis of their motility.

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Abbreviations:
ECF-A: eosinophil chemotatic factor of anaphylaxis
HMW-NCF: high molecular weight neutrophil chemotactic factor

RECOGNITION AND PARTIAL PURIFICATION OF HMW-NCF

A factor chemotactic for neutrophilic polymorphonuclear leukocytes increased in amount in the venous effluent of the cold-immersed arm in each of 7 patients with idiopathic acquired cold-induced urticaria. The neutrophil chemotactic activity was noted by 30 seconds, reached peak levels at 2 min, and was dissipated by 15 min (Fig 1). Normal individuals subjected to cold immersion did not develop augmented chemotactic activity in the venous effluent of the challenged arm. Assessment of the complement system, in terms of C1, C4, C2, C3, and C9 functional activity and C1q, C4, C3, C5, factor B, properdin, and $C\bar{1}$ inhibitor protein concentrations, showed no differences between the normal and challenged arms; there were no alterations from the baseline values.

The augmented neutrophil chemotactic activity was partially purified by sequential chromatographic procedures. Sephadex G-200 gel filtration of paired serum specimens with peak chemotactic activity from the lesional and control arms showed augmented neutrophil chemotactic activity in the lesional serum that filtered in the void volume. This activity was further purified by QAE-Sephadex chromatography, eluting between 6 and 12 mS, and by SP-Sephadex chromatography, where it appeared in the effluent. The chemotactic activity was excluded on Sepharose 4B chromatography, indicating a molecular weight in excess of 750,000. On isoelectric focusing the partially purified neutrophil chemotactic factor exhibited a neutral isoelectric point when focused in 2 different support media with single peaks of focus between pH 6.7 and 7.4 (Fig 2).

HMW-NCF AND THE MOTILITY OF NEUTROPHILIC LEUKOCYTES *IN VITRO*

The effect of this factor on the motility of neutrophilic leukocytes was studied *in vitro* and compared with the responses of mononuclear leukocytes. The partially purified high molecular weight factor attracted neutrophilic leukocytes in a dose-response fashion at concentrations that had little or no effect on mononuclear leukocytes (Fig 3). The neutrophil response was abrogated if the concentration gradient was abolished by placing identical concentrations of the factor on the cell and stimulus sides, indicating that the active principle was a chemotactic factor.

In addition to attracting target cells, chemotactic factors render these cells unresponsive to subsequent chemotactic responses, an action termed deactivation. HMW-NCF deactivated neutrophils in a time- and dose-related fashion; the concentrations effective in deactivation were subthreshold to minimal compared to the chemotactic stimulus (Fig 4).

NEUTROPHILIC LEUKOCYTE FUNCTION *IN VIVO*

Since chemotactic principles were released into the circulation, the nature of the local lesional tissue alterations was examined. Skin biopsy specimens of the experimental angioedematous lesions obtained over 24 hr showed early mast cell degranulation with venular endothelial cell alterations but no

infiltrating polymorphonuclear leukocytes of any class [8]. The absence of infiltrating cells in the angioedematous lesion after a single challenge is due presumably to an action of the large amounts of released chemical mediators on the motility of the cells.

The migration of neutrophilic leukocytes obtained from subjects prior to and at time intervals after ice challenge and subsequent exposure to mediators *in vivo* was assessed *in vitro*. Neutrophilic leukocytes harvested from the challenged arm at 5 min were markedly impaired in their chemotactic responses to HMW-NCF (Fig 5). The neutrophil defect in the cells from the challenged arm was attributed to mast cell activation and the release of mast cell mediators in the angioedematous skin since neither chemical mediators nor chemotactic deactivation of circulating cells was found in the unchallenged arm at the same time. The local release of mediators also had a systemic

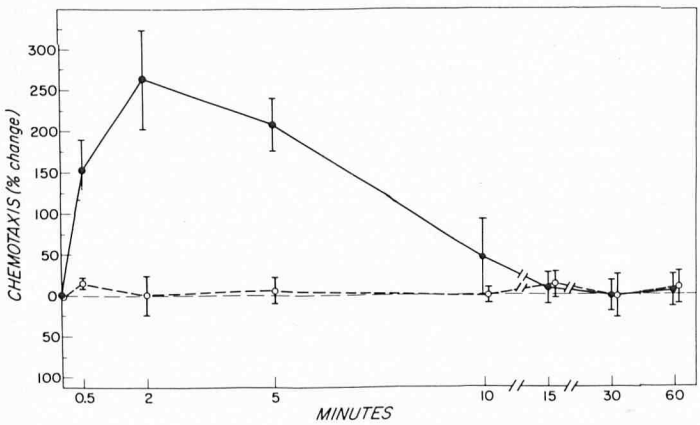

FIG 1. Time-course of appearance of neutrophil chemotactic activity in the venous effluent sera from 7 subjects after experimental ice challenge. Each *point* represents the mean (± 1 SD) net percent change in the neutrophil chemotactic activity in lesional (●) and control (○) arms, as compared to time zero. From reference 1.

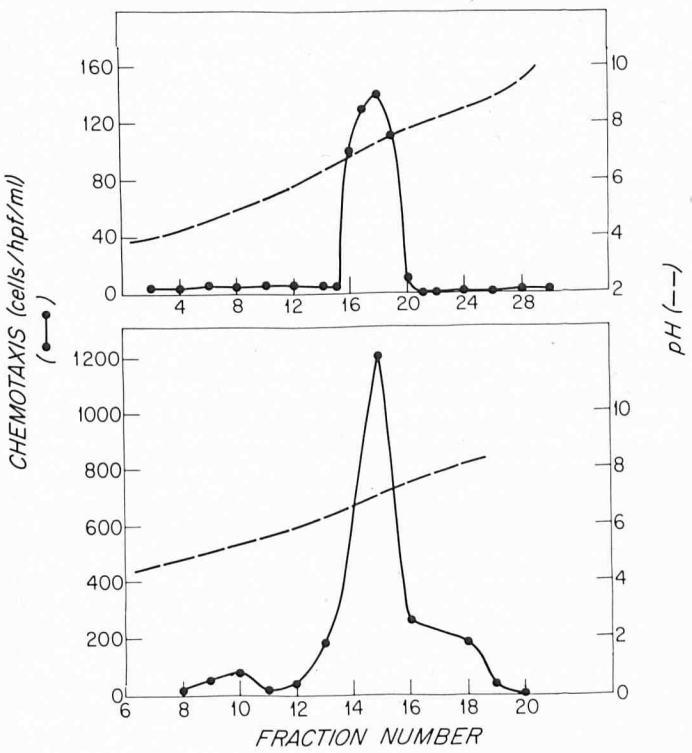

FIG 2. Isoelectric focusing in sucrose (*upper*) and in thin-layer Sephadex G-75 (*lower*) of partially purified HMW-NCF. From reference 1.

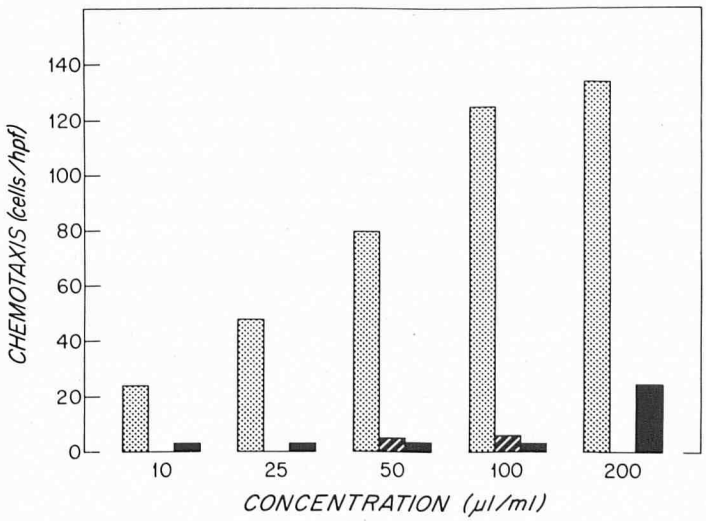

FIG 3. Chemotactic responses of neutrophilic leukocytes (*stippled bars*) and mononuclear leukocytes (*solid bars*) to the purified HMW-NCF. The effect of eliminating the gradient by placing chemotactic factor in both compartments of the chamber is depicted by *hatched bars*. From reference 1.

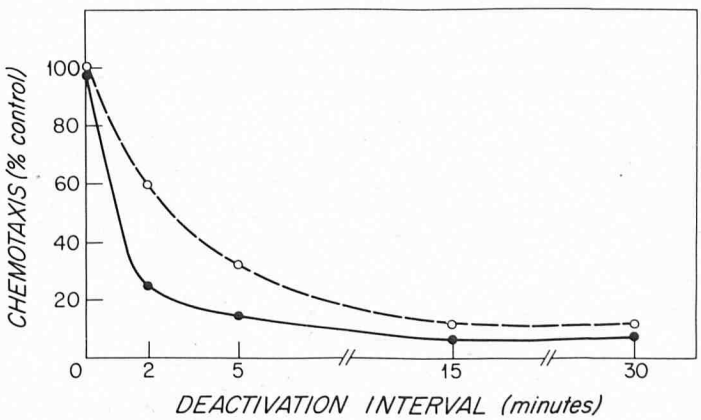

FIG 4. Time-dependent deactivation of neutrophilic leukocytes by 5 (○) or 25 (●) μl of HMW-NCF as assessed by their subsequent chemotactic response to 100 μl of the same stimulus. From reference 1.

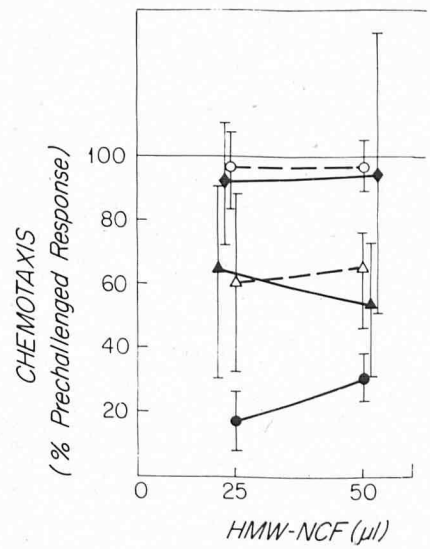

FIG 5. Mean percentage chemotactic response of neutrophilic leukocytes as compared to response of cells obtained prior to cold challenge. Control arm at 5 min (○) and 60 min (△); cold-challenged arm at 5 min (●), 60 min (▲), and 4 hr (◆). *Vertical bars* show range. The stimulus was HMW-NCF. From reference 2.

effect on neutrophils, as demonstrated by the impaired chemotactic function of cells from both challenged and unchallenged arms at 60 min. A systemic effect on neutrophil function is presumably due to the circulation of the neutrophilic leukocytes through the cold-challenged arm and their subsequent dispersion throughout the circulation. The chemotactic abnormality was no longer present at 4 hr. Deactivation would account satisfactorily for the rapid onset of the neutrophil defect in the cold-challenged arm, its later systemic distribution, and the absence of cells in the angioedematous tissue lesion.

DISCUSSION

A neutrophil chemotactic activity (HMW-NCF) was recognized in sera of patients with idiopathic cold-induced urticaria after local experimental cold challenge with ice. The time-course of the appearance of this serum chemotactic activity was indistinguishable from that of histamine and a low-molecular weight eosinophil chemotactic factor, implying a mast cell origin for this mediator. Chemotactic activity preferential for neutrophilic leukocytes and greater than 30,000 molecular weight has previously been noted in extracts of human leukemic basophils [9] and lung fragments [10], but these activities have not been further characterized. The appearance of augmented neutrophil chemotactic activity in the serum of asthmatic patients after inhalation challenge with specific antigen has also been reported [11, 12]. In studies of mediator release into the circulation in patients with the IgE-mast cell-mediated disorders cholinergic urticaria [13] and solar urticaria [14], a neutrophil chemotactic factor has been detected. Taken together, the studies in both physical urticarias and asthmatic patients [11, 12, 15] suggest that neutrophil chemotactic activity may be a marker for mast cell activation *in vivo*.

The observation that neutrophils are rendered less responsive to chemotactic stimuli *in vitro*, after exposure to a chemotactic stimulus *in vivo*, suggests that deactivation could be a regulatory mechanism for limiting the local tissue influx of inflammatory cells. It could also diminish general host responsiveness if the release of chemotactic factors was persistent or excessive.

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DISCUSSION

TIGELAAR: Is the deactivation of neutrophil chemaotaxis that you have described specific, in the sense that pre-exposure to HMW-NCF leads to unresponsiveness only to HMW-NCF and not to, for example, C5a?

Soter: We have examined one other chemotactic factor, the tryptic digest of C5, on these cells exposed to high molecular weight NCF *in vivo*. The cells exhibit the deactivation phenomenon, so there is a crossdeactivation.

BERGSTRESSER: Since this high molecular weight chemotactic factor found in these patients is released in the absence of a neutrophilic infiltrate, what do you believe the functional role of the factor to be?

SOTER: I think it's the experimental circumstance that would determine the functional role. It is conceivable that in some inflammatory conditions in which the mast cell is involved, this factor could allow cells to enter the tissues. In this case there is a sudden massive release that is overwhelming the cells in the circulation preventing their entry into tissue sites.

KATZ: In that regard, have you injected it?

SOTER: No.

RAY: Will NCF-induce lysosomal enzyme release of beta-glucoronidase or leucine amino peptidase activity as noted with other chemoattractants?

SOTER: We do not find that it is a model of other classes of chemotactic factors. For example, the chemotactic factors derived from the arachidonic pathway do not release lysosomal enzymes. Similar observations were noted with the tetrapeptide ECF-A.

GIGLI: Do you think that the size of this factor would indicate that it may be a complex of 2 proteins?

SOTER: This is a possibility but it is not yet known.